

**P006** Sorting of *trans*-membrane domain proteins from GPI-anchored protein requires ubiquitylation in trypanosomes  
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In bloodstream form *Trypanosoma brucei*, the surface is dominated by glycosylphosphatidylinositol (GPI) anchored proteins, mainly the variant surface glycoprotein (VSG). Endocytosis is strictly clathrin-dependent and the vast majority of internalized GPI-anchored protein is efficiently recycled. The processes by which *trans*-membrane domain (TMD) proteins, such as ISG65 and ISG75, are internalized and sorted are unknown. Using a reporter protein BiPNTm, which contains the TMD and cytoplasmic domain of ISG65, we demonstrate that internalization and turnover of ISG65 is dependent on position-specific cytoplasmic lysines. This was also observed when the ISG65 sequence was replaced with ISG75. In addition, we detect the presence of ubiquitylated forms of BiPNTm in a lysine-dependent fashion. Ubiquitylation is context-dependent, as provision of additional lysine residues by C-terminal attachment of NEDD8 failed to support ubiquitylation. Interestingly, attachment of NEDD8 to BiPNTm leads to degradation by a second, ubiquitin-independent, pathway. RNAi of two E1 ubiquitin-activating enzymes did not affect ISG65 turnover while E1 double knockdown was lethal. Furthermore, knockdown of clathrin inhibits endogenous ISG65 internalization while knockdown of the ESCRT factor, TbVps23, partially prevents ISG65 degradation. Thus, ubiquitin-dependent internalization of surface proteins appears well conserved across evolution, albeit with some differences between the major eukaryotic lineages.